An Analytical Profile of Aceclofenac

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ABSTRACT: The analysis and characterization of aceclofenac (2-[(2,6-dichlorophenyl)amino]phenyl-acetoxyacetic acid) by GC/MS, ESI-Ion Trap-MS, UHPLC-QTOF-MS, LC-SQD-MS, FTIR/ATR, and NMR is presented and discussed.

KEYWORDS: Aceclofenac, Diclofenac, 1-(2,6-Dichlorophenyl)-2-indolinone, Characterization, Forensic Chemistry.

Introduction

Figure 1

This laboratory recently received an exhibit of oblong, pink colored tablets (Figure 1), half-scored on one face and blank on the opposite face, suspected to be Percocet™ (i.e., an oxycodone/acetaminophen preparation). The tablets had been purchased from an India-based internet pharmacy. Preliminary analysis, however, indicated that the tablets actually

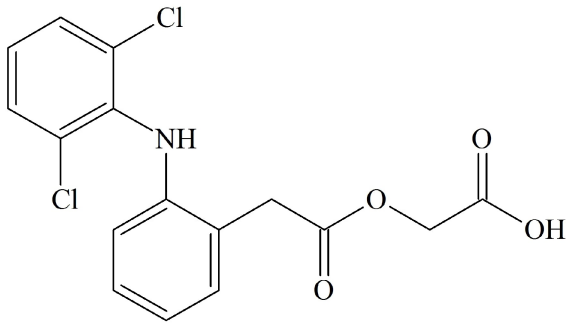

Figure 2 - Aceclofenac
($C_{16}H_{13}Cl_2NO_4$, mw = 354.185)

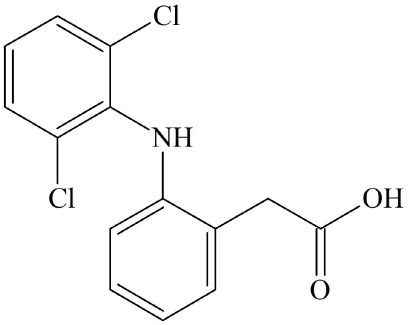

Figure 3 - Diclofenac
($C_{14}H_{11}Cl_2NO_2$, mw = 296.148)

contained a mixture of aceclofenac (2-[(2,6-dichlorophenyl)amino]phenylacetoxy-acetic acid, Figure 2) and acetaminophen. Aceclofenac is the glycolic (hydroxyacetic) acid ester of diclofenac (Figure 3) and is a non-steroidal anti-inflammatory drug (NSAID) with analgesic properties, typically utilized for osteoarthritis and similar afflictions (1-3). It is not available over-the-counter in the U.S., but can be easily purchased through international internet pharmacies. It is not controlled under any U.S. or state statutes at the present time, but is a prescription medication in the U.K., Italy, Spain, and elsewhere. It is widely utilized in India and other south Asian nations as a substitute for diclofenac, due to the latter drug's unacceptable environmental impacts, most notably its lethal effects on vultures (4). Its abuse potential is considered to be low.

Due to the facile loss of glycolic acid in heated injection ports during standard GC/MS analysis, aceclofenac does not display a molecular ion, but rather a pseudomolecular ion at *m/z* 277, representing a thermal decomposition product. Diclofenac similarly loses water during standard GC/MS analysis, elutes at a nearly identical retention time, also displays a pseudomolecular ion at *m/z* 277, and displays a highly similar fragmentation pattern, indicating formation of the same breakdown product in heated injection ports (Figures 4 and 5). Therefore, GC/MS cannot be utilized to unambiguously identify or differentiate either compound.

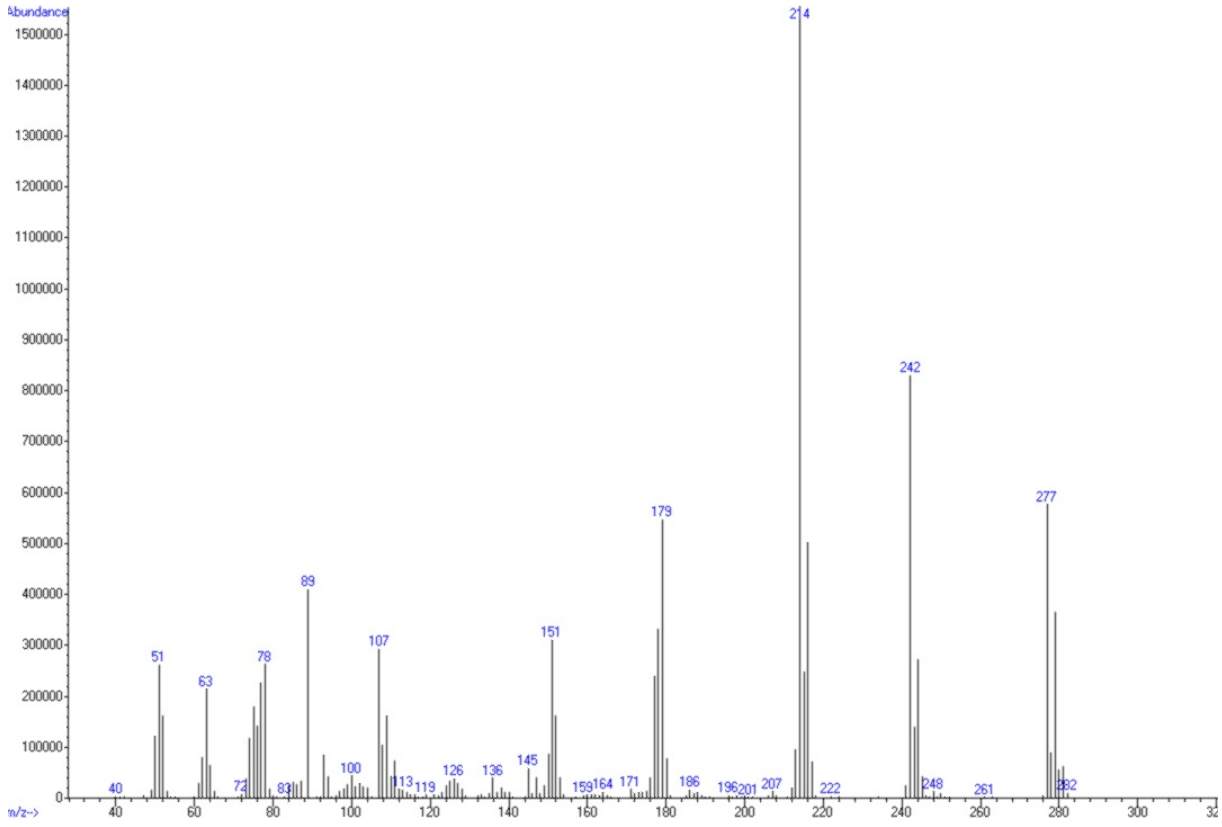

Figure 4 - GC/MS Spectrum of Aceclofenac (Molecular Ion Not Detectable)

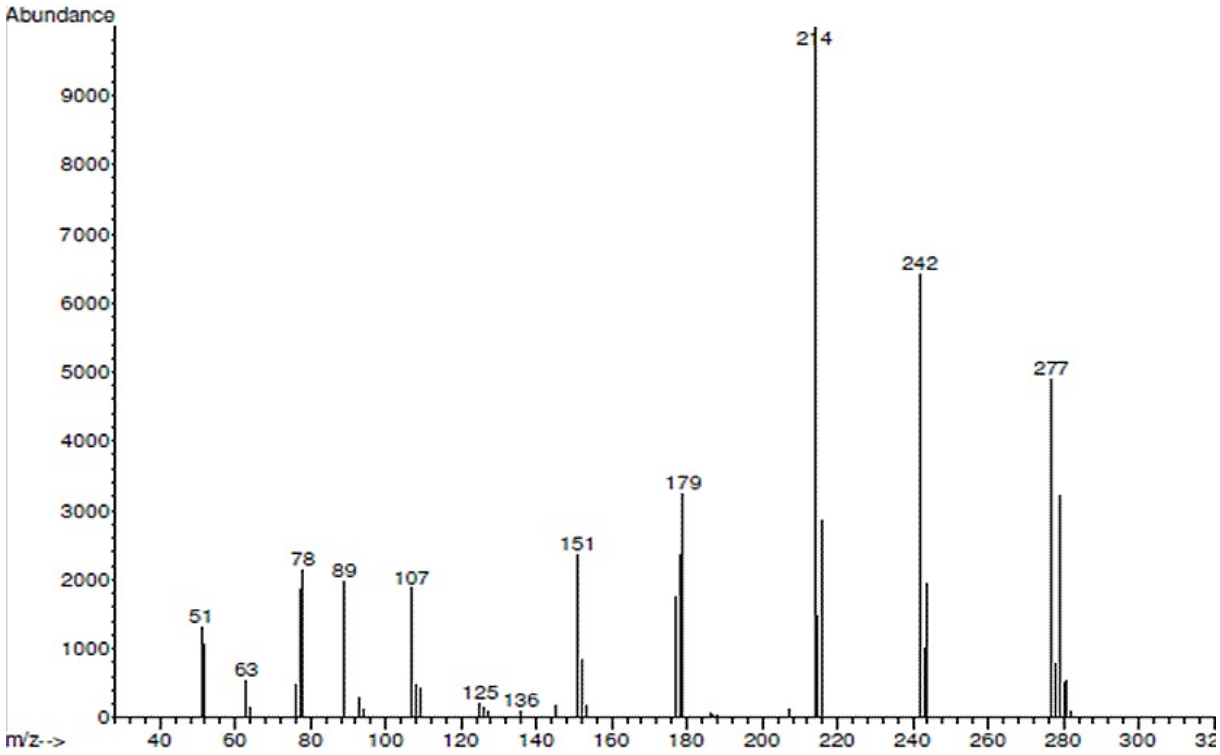

Figure 5 - GC/MS Spectrum of Diclofenac (Molecular Ion Not Detectable)

Previous reports have presented the characterization of aceclofenac: By mp, ^{1}H- and ^{13}C-NMR, IR, and elemental analysis (5); by mp, UV λ_{max}, FTIR, NCI-MS, and ^{1}H-NMR (6); by ^{1}H and ^{13}C NMR (7); by UPLC-QTOF-MS and UPLC-QTOF-MS/MS (8); by mp (by DSC), UV/Vis, FTIR (in KBr), UPLC-QTOF-MS, and UPLC-QTOF-MS/MS (9); by FTIR (in KBr), FT-Raman, and UV/Vis (10); by mp (by DSC), UV/Vis (in 0.1N HCl), and FTIR (11); by mp (by DSC), FTIR (in KBr), and XRD (12); by FTIR and XRD (13); by mp and FTIR (in KBr) (14,15); and by UV/Vis (16-21); however, much of the published data is of lower quality, lacks experimental details, or is published in rather obscure venues that can be challenging for forensic analysts to access. Herein the characterization of aceclofenac by GC/MS, ESI-Ion Trap-MS, UHPLC-QTOF-MS, LC-SQD-MS, FTIR/ATR, and ^{1}H, ^{13}C, and ^{15}N NMR, is presented and discussed.

Experimental

Chemicals, Reagents, and Materials

Aceclofenac and diclofenac were obtained from Sigma Aldrich. LC-grade water was obtained from a Millipore filter. LC-grade methanol, acetonitrile (ACN), and pre-mixed solutions containing 0.1 % formic acid, were obtained from J.T. Baker.

Gas Chromatography/Mass Spectrometry (GC/MS)

Analyses were conducted on an Agilent (Santa Clara, CA) Model 7890A gas chromatograph coupled to an Agilent Model 5975C single quadrupole mass-selective detector (MSD). The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm HP-5 (Agilent). The oven temperature was programmed as follows: Initial temperature 90^{O}C; initial hold 1.35 minutes; program rate

35°C/minute to 120°C, hold 0.55 minutes; ramp 45°C/minute to a final temperature of 290°C, final hold 8.5 minutes. The injector was operated in the split mode (50:1) at 280°C. The carrier gas was Helium. The MSD was operated in the EI mode at 70 eV, with a scan range of 40-550 amu and a scan rate of 2 scans/second. The MSD source was operated at 230°C.

Electrospray Ionization - Ion Trap - Mass Spectrometry (ESI-Ion Trap-MS)

Tandem mass spectral analyses were conducted on a Thermo Scientific (Waltham, MA) Accela HPLC coupled to a Thermo Scientific LXQ ion trap MS (this instrument has MS^N capabilities). The sample was injected directly into the spectrometer (i.e., with no chromatographic separation) using ACN (w/ 0.1% formic acid) / Millipore water (w/ 0.1% formic acid) 1:1, delivered at 0.200 mL/minute (i.e., the HPLC was only utilized to automate the injection). The spectrometer was operated in the positive ESI mode, with a scan range of 50 - 500 amu and a collision-induced-dissociation (CID) of 25 normalized energy units. The nitrogen sheath gas was at 40.0 Arb; the aux/sweep gas at 5.0 Arb; the capillary voltage at 4 kV; and the capillary temperature at 350°C.

Ultra-High Performance Liquid Chromatography - Quadrupole-Time of Flight Mass Spectrometry (UHPLC-QTOF-MS)

High-resolution mass spectral analyses were conducted on an Agilent 1290 Infinity UHPLC interfaced with an Agilent 6520 QTOF-MS. The UHPLC was fitted with a Zorbax Extend-C18 column, 2.1 mm x 50 mm, with a 1.8 µm particle size, operating at 40°C. A gradient mobile phase was utilized: Initial 30% ACN (w/ 0.1% formic acid) / 70% Millipore water to final 70% ACN (w/ 0.1% formic acid) / 30% Millipore water over 8 minutes, delivered at 0.5 mL/minute. The spectrometer was operated in the positive ESI mode with the capillary voltage at 4 kV. The nitrogen drying gas temperature was 350°C with a flow rate of 13 L/minute. The nebulizer pressure was set to 50 psi. The fragmentor voltage was set at 150 V; the skimmer voltage at 60 V; and the octopole 1 Rf Vpp at 750 V. Internal mass calibration was achieved using two reference mass ions at *m/z* 121.0509 and 922.0098. The mass accuracy was calculated to be 0.31 ppm.

Liquid Chromatography - Single Quadrupole - Mass Spectrometry (LC-SQD-MS)

Low-resolution mass spectral analyses were conducted on an Agilent 1200 series binary pump LC interfaced with an Agilent 6130 single quadrupole MSD (SQD-MS). The LC was fitted with an Agilent Zorbax Eclipse XDB C18 column, 4.6 mm x 50 mm, with a 1.8 µm particle size, operating at 40°C. The isocratic mobile phase was 65% ACN (with 0.1% formic acid) / 35% Millipore water, delivered at 0.75 mL/min. Nitrogen was used as both the nebulizer and drying gas. The MSD was operated in the positive ESI mode with the capillary voltage at 4 kV, drying gas flow rate of 13 L/min, nebulizer pressure of 50 psi, and a drying gas temperature of 350°C. The fragmentor voltages were set at 100 V for the first analysis and 150 V for the second analysis.

Fourier Transform Infrared / Attenuated Total Reflectance Spectroscopy (FTIR/ATR)

A Perkin Elmer (now Thermo Scientific, Waltham, MA) Frontier FTIR equipped with an ATR accessory was utilized. The spectrum was acquired using 8 scans at 4 cm^{-1} resolution, from 4000 to 650 cm^{-1}.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Aceclofenac was prepared at 15 mg/mL in dimethyl sulfoxide-*d*6 containing 0.05% v/v TMS

(Cambridge Isotope Laboratories, Andover, MA). ^{1}H, ^{13}C, HSQC, HMBC, COSY and 1D TOCSY spectra were acquired on an Agilent VNMRS 600 MHz NMR using a broadband probe (^{1}H parameters: 8 scans, 45^{O} pulse width, 45 s delay between pulses, and 5 s acquisition time). ^{15}N-HSQC and ^{15}N-HMBC spectra were acquired on an Agilent VNMRS-DD2 400 MHz NMR using an OneNMR probe. All spectra were collected with a sample temperature of $25^{O}C$. Spectra were processed using ACD/Structure Elucidator, version 14.01, Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada.

Results and Discussion

Somewhat unexpectedly, there do not appear to be any previous literature reports concerning the GC/FID or GC/MS analyses of aceclofenac - doubtless due to its facile degradation in heated injection ports. As noted in the Introduction, the standard GC/MS spectra of aceclofenac and diclofenac are markedly similar, with nearly identical retention times (approximately 6.07 minutes on the described system), identical pseudomolecular ions, and highly similar fragmentation patterns (differing only moderately in relative abundances), confirming formation of a common breakdown product.

From a mechanistic viewpoint, the loss of glycolic acid from aceclofenac and water from diclofenac would generate either a ketene (i.e., 2-(2,6-dichlorophenyl)amino-phenyl ketene, Figure 6) from abstraction of the *alpha* (benzylic) proton during de-esterification / dehydration, or an indolinone (i.e., 1-(2,6-dichlorophenyl)-2-indolinone, Figure 7) from intramolecular displacement of the ester or hydroxyl groups by the amine, resulting in lactamization). In fact, the mass spectrum of 1-(2,6-dichlorophenyl)-2-indolinone (22-24) is quite similar to the mass spectra of aceclofenac and diclofenac, confirming the latter mechanism. Interestingly, the mass spectra of the methyl and ethyl esters of diclofenac display their respective molecular ions at significant relative abundances (indicating greater thermal stability), but are otherwise also quite similar to aceclofenac and diclofenac (24,25).

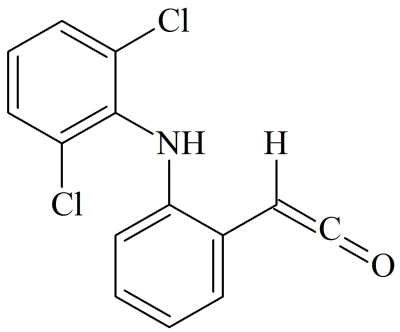

Figure 6 - 2-(2,6-Dichlorophenyl)amino-phenyl ketene [Note: Probably not a stable compound]

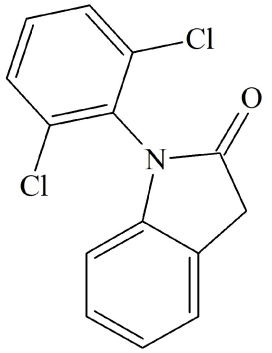

Figure 7 - 1-(2,6-Dichlorophenyl)-2-indolinone

With GC/MS unable to unambiguously differentiate aceclofenac, diclofenac, 1-(2,6-dichlorophenyl)-2-indolinone, and potentially other diclofenac esters, additional mass spectral analyses of aceclofenac were conducted. ESI-Ion Trap-MS confirmed the molecular ion in single-stage/full-MS mode at *m/z* 354.00 (Figure 8a). The second-stage (MS^{2})

fragmentation indicated a breakdown from the molecular ion into two fragments at *m/z* 277.93 and *m/z* 250.00, both resulting from the cleavages of the acetoxyacetic acid substituent (Figure 8b). The third-stage (MS^3) fragmentation indicated the loss of one chloro substituent from the fragment at *m/z* 250.00, giving a fragment at *m/z* 215.04 (Figure 8c).

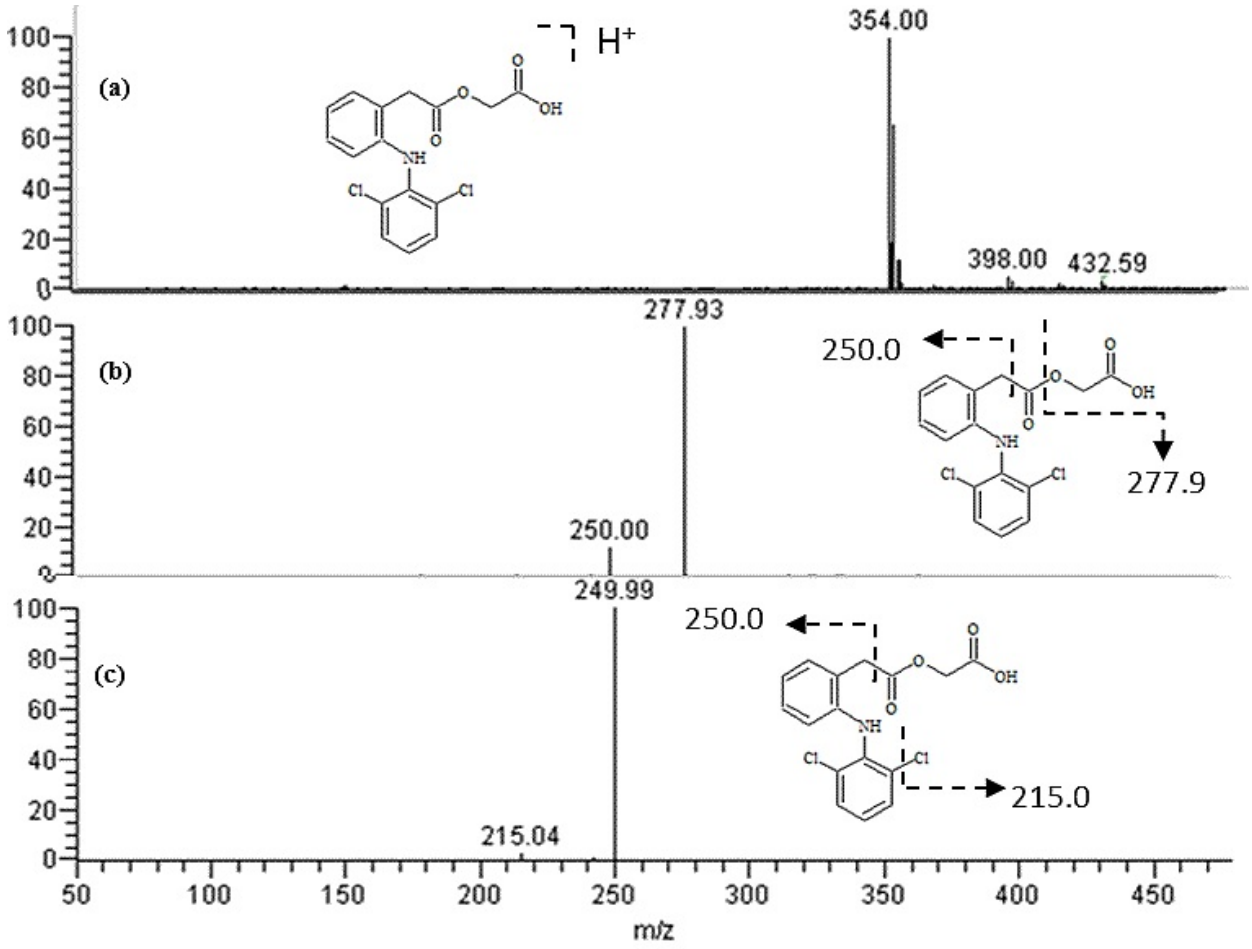

Figure 8 - ESI-Ion Trap-MS of Aceclofenac: (a) Full Scan Mass Spectrum; (b) Fragmentation of the Pseudomolecular Ion at *m/z* 354 (MS^2); (c) Fragmentation of the Product Ion at *m/z* 277.9 (MS^3).

High-Resolution QTOF-MS confirmed the molecular ion at *m/z* 354.0293, corresponding to $C_{16}H_{14}Cl_2NO_4$ (i.e., the protonated pseudomolecular $[M+H]^+$ ion; Figure 8). The sodiated adduct ($C_{16}H_{13}Cl_2NO_4Na^+$) was observed at *m/z* 376.0110 (Figure 9).

Finally, low-resolution SQD-MS at 100 V (a moderately low collision-induced energy) again confirmed the molecular ion at *m/z* 354.1 (Figure 10a). At 150 V, three product ions were observed at *m/z* 278, 250, and 215, complementing the results obtained in the tandem MS analyses (Figure 10b).

Each of these three positive ESI mass spectral analyses easily differentiate aceclofenac versus diclofenac or any common diclofenac ester.

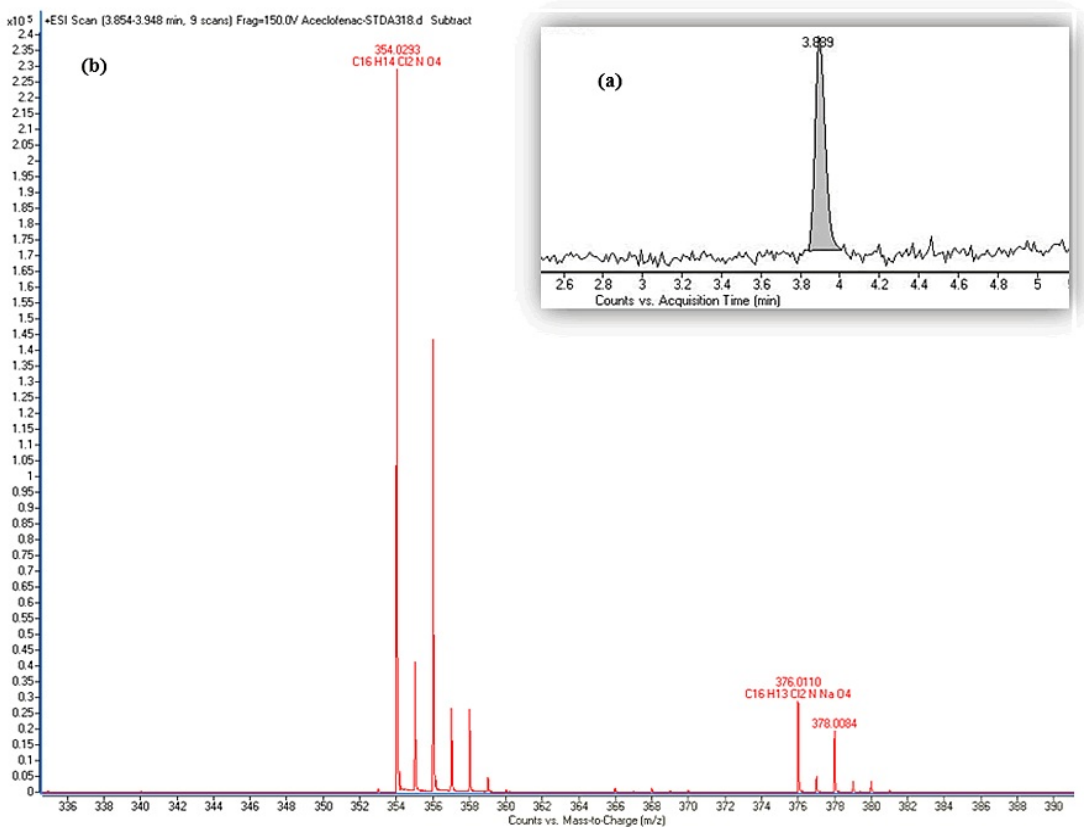

Figure 9 - UHPLC-QTOF-MS of Aceclofenac: (a) TIC (UHPLC Retention Data Reported in Table II); (b) High Resolution Mass Spectrum Including the Calculated Empirical Formula for the Pseudomolecular Ion and its Sodiated Adduct.

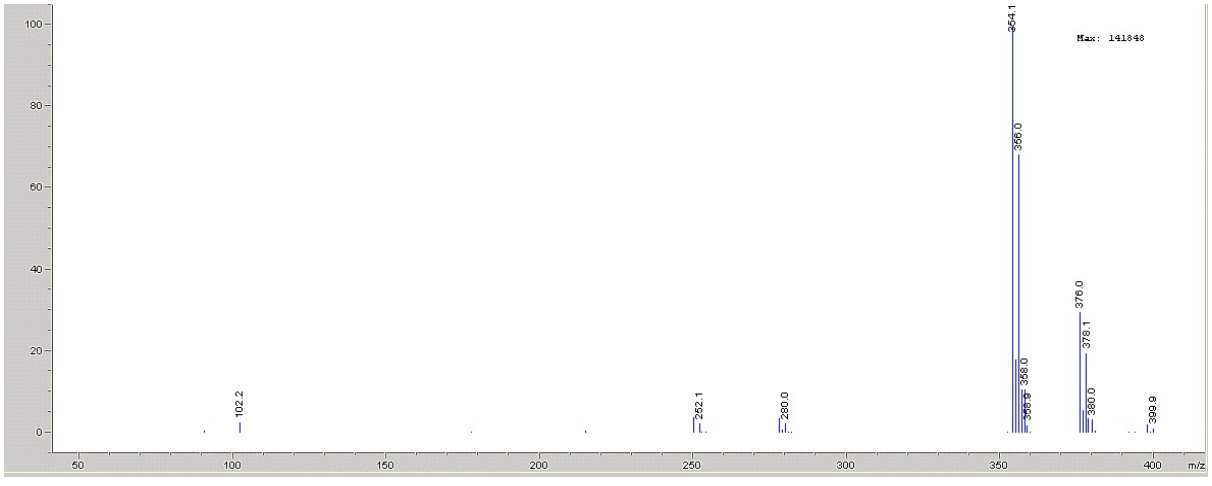

Figure 10a - SQD Mass Spectrum of Aceclofenac at 100 V (LC Retention Data Reported in Table 1).

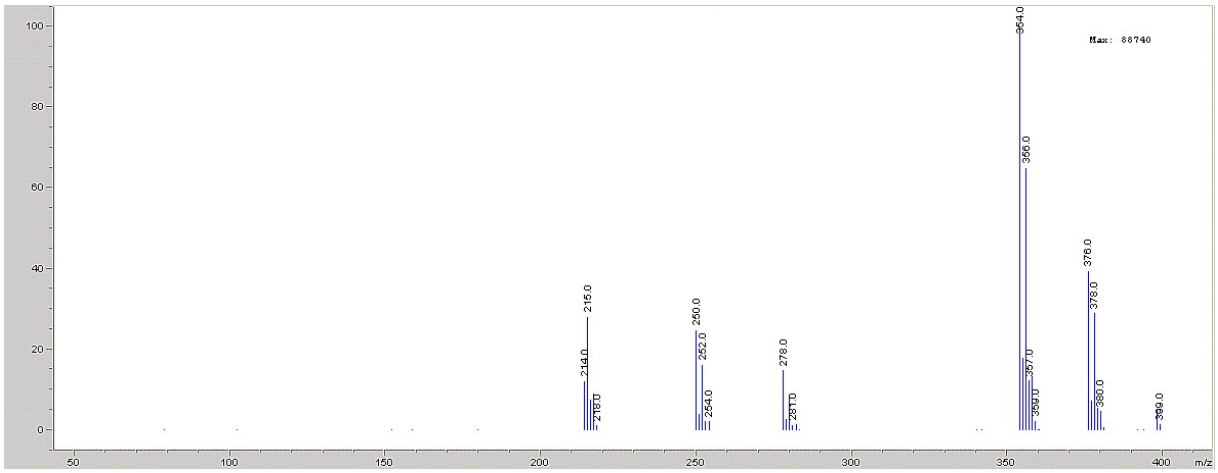

Figure 10b - SQD Mass Spectrum of Aceclofenac at 150 V (LC Retention Data Reported in Table 1).

The FTIR/ATR spectrum (Figure 11) is unremarkable, with a broad secondary amine and carboxylic acid (hydroxyl) band from 3200 and 3400 cm^{-1}, two ketone bands at 1716 and 1771 cm^{-1}, and multiple, moderate to prominent phenyl ring bands from 650 to 1600 cm^{-1}. The spectrum is more than adequate for identification of high purity samples.

The ^{1}H-NMR spectrum is presented in Figure 12. Chemical shift, peak shape, and correlation information, in conjunction with ACD/Structure Elucidator, were used for structure elucidation. The ^{1}H, ^{13}C, and ^{15}N assignments are reported in Table 2 (the ^{13}C, HSQC, HMBC, COSY, and 1D TOCSY spectra are not shown). Proton and carbon chemical shifts are referenced to TMS,

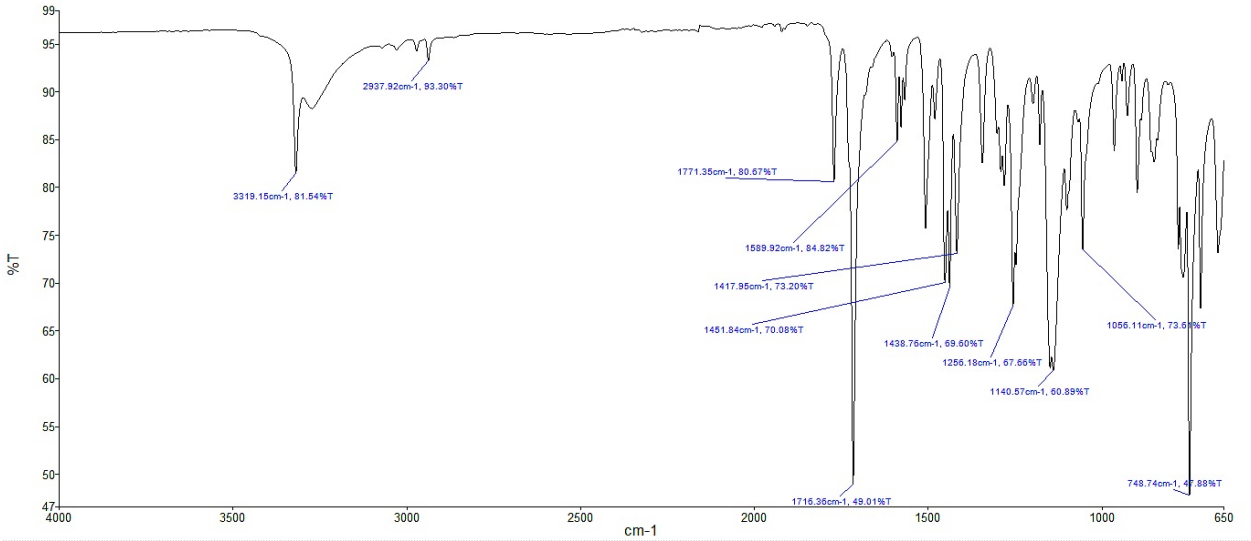

Figure 11 - FTIR/ATR Spectrum of Aceclofenac.

while the nitrogen value is unreferenced. The most notable difference between the ^{1}H-NMR spectra of aceclofenac and diclofenac is aceclofenac's additional singlet at 4.64 ppm, from the glycolic ester methylene group. The results easily differentiate aceclofenac versus diclofenac or any common diclofenac ester.

Acknowledgments

I would like to thank everyone who has taken the time to review this analytical profile, in particular Senior Forensic Chemist Yuriy Uvaydov and Supervisory Chemist Ann Marie O'Neill (both of this laboratory) for their constructive comments and guidance. I would also like to thank Senior Forensic Chemists Charlotte Corbett and Roxanne Franckowski of the DEA Special Testing and Research Laboratory (Dulles, VA) for their assistance with the NMR analyses and structural elucidation, and additionally to Charlotte Corbett for providing the structures for Figures 2, 3, 6, and 7, and within Table 1.

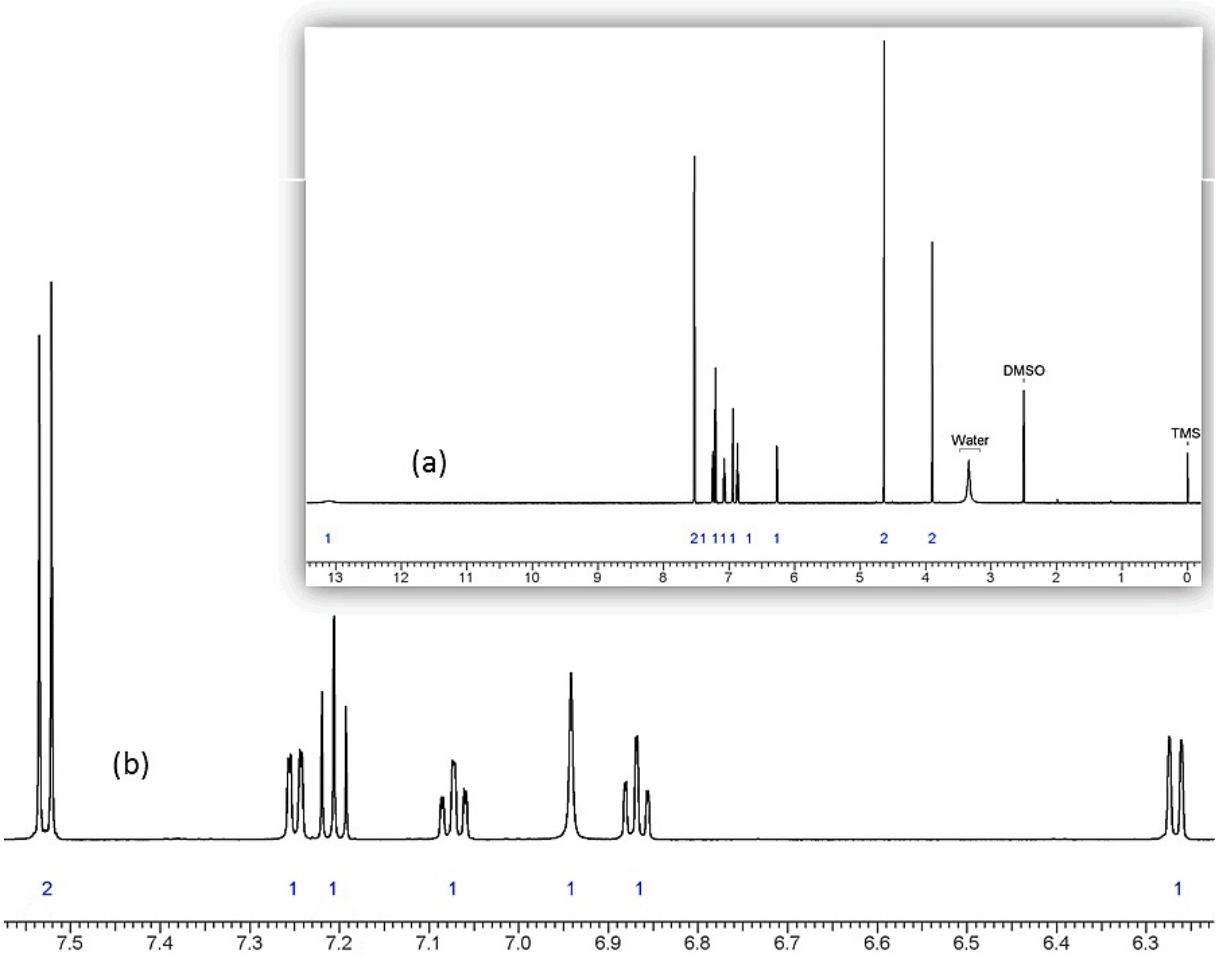

Figure 12 - (a) 600 MHz ^{1}H-NMR Spectrum of Aceclofenac;
(b) Expansion between 6.1 - 7.7 ppm.

Compound	UHPLC	LC
Aceclofenac	3.94 minutes	1.52 minutes
Diclofenac	3.73 minutes	1.56 minutes

Table 1 - Retention Data for Aceclofenac and Diclofenac
(Details Provided in the Experimental Section).

Position	^{13}C or ^{15}N (ppm)	^{1}H (ppm)	^{1}H multiplicity and J_{HH} (Hz)
1	123.0	-	-
2	142.8	-	-
3	116.0	6.27	d 7.8
4	127.7	7.07	td 7.6, 1.4
5	120.7	6.87	td 7.5, 1.1
6	130.8	7.25	dd 7.6, 1.3
NH	128.6	6.94	s
1'	137.1	-	-
2'	130.6	-	-
3'	129.0	7.53	ad 8.1
4'	125.9	7.21	t 8.1
5'	129.0	7.53	ad 8.1
6'	130.6	-	-
-**CH_2**$COOCH_2COOH$	36.6	3.90	s
-CH_2**C**$OOCH_2COOH$	170.9	-	-
-CH_2COO**CH_2**$COOH$	61.0	4.64	s
-CH_2COOCH_2**COOH**	168.9	13.11	brs

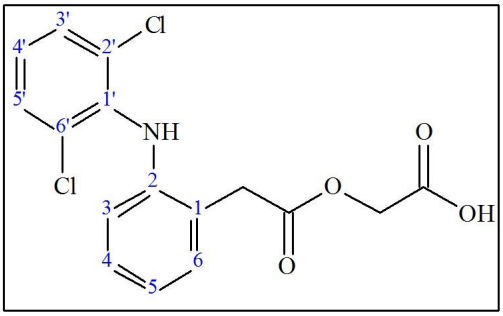

Table 2 - Structural Assignments obtained via NMR (Side Figure Shows the Numbering Protocol).
[a= apparent, d = doublet, s = singlet, t = triplet, br = broad]

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7. ^{1}H and ^{13}C NMR of aceclofenac: http://www.molbase.com/en/hnmr_89796-99-6-moldata-6639.html [Date of most recent access: 6/24/2015.] [Note: This is a commercial vendor's website; the source of the spectra (which are of good quality) is unknown, and the field strengths are not specified. It is routine for such sites to reprint literature data on their websites, without attribution.]

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